

**Effect of fat and salt reduction on the sensory quality of slow fermented
sausages inoculated with *Debaryomyces hansenii* yeast**

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Abstract

The inoculation of a *Debaryomyces hansenii* strain in dry fermented sausages with reduced fat and salt contents was evaluated in terms of chemical, microbial and consumer acceptability. The implantation of the inoculated yeast strain was confirmed by RAPDs of M13 minisatellite. A reduction of 17-20 % salt and 10-16 % fat content was achieved. These reductions affected the sausage quality by producing an increase in a_w , hardness and chewiness values and a decrease of staphylococci growth. However, *D. hansenii* inoculation compensated these changes although it was not able to modify **neither** the hardness of reduced fat batches **nor** the staphylococci growth decrease. In terms of sensory acceptability, different preferences patterns of consumers were found. Yeast inoculation improved the aroma and taste quality when fat or salt reductions were carried out in dry fermented sausages.

Keywords: fat, salt, sensory, *Debaryomyces hansenii*, dry fermented sausage, yeast.

1. Introduction

The intake of meat products has grown around 10% in industrialized countries in spite of their fat and salt content (WHO/FAO, 2003). However, during the last years a demand for low fat and salt food has grown (Ruusunen & Puolanne, 2005). Therefore, meat products such as dry fermented sausages are being reformulated to adjust their fat and salt content (Aaslyng, Vestergaard & Koch, 2014; Beriain, Gómez, Petri, Insausti & Sarriés, 2011). However, these ingredients cannot be reduced without affecting organoleptic and technologic characteristics. On the one hand, fat contributes to nutritional (source of essential fatty acids, liposoluble vitamins and energy), organoleptic (flavour, texture, mouthfeel) and technological properties (release of moisture) (Olivares, Navarro, Salvador & Flores, 2010). On the other hand, salt is also involved in organoleptic (flavour, texture) and technologies properties (myofibrillar protein solubilization, a_w decrease) (Corral, Salvador & Flores, 2013).

Different strategies have been studied to reduce fat and salt content in dry fermented sausages, since preservation of product acceptability is a decisive criterion when developing this kind of products (Wirth, 1988). Fat content has been replaced by soy oil (Muguerza, Ansorena & Astiasarán, 2003), olive oil (Bloukas, Paneras & Fournitzis, 1997), konjac gel (Ruíz-Capillas, Triki, Herrero, Rodriguez-Salas & Jiménez-Colmenero, 2012), inulin (Mendoza, García, Casas & Selgas, 2001), and fibres (Salazar, García, & Selgas, 2009). However, few studies have dealt with fat reduction without replacers and its effect on sensory characteristics. Generally, fat reduction (10 %) affected the external appearance and flavour intensity of fermented sausages (Liaros, Katsanidis & Bloukas, 2009) while higher reduction percentages (20 %) produced a suitable acceptability (Papadima & Bloukas, 1999 and Olivares et al. 2010); in spite of a lowest taste, aroma and hardness (Olivares et al., 2010 and 2011) and appearance (Papadima & Bloukas, 1999) perceived by consumers.

Regarding salt reduction, different salts have been used as NaCl substitute (Corral et al., 2013; Gimeno, Astiasarán & Bello, 1999) although they did not achieve

sensory acceptable products (Gimeno et al., 1999). Major salt reductions (40-50%) had a negative effect on many sensory characteristics such as hardness, bitterness, aroma and taste acceptability (Gelabert, Gou, Guerrero & Arnau, 2003; Campagnol, dos Santos Wagner, Terra & Pollonio, 2011). With small salt reductions (16 %), the best results were found when KCl alone was used even though the aroma acceptability was still affected (Corral et al., 2013).

The effect of fat and salt reductions together in dry fermented sausages has been scarcely studied. A strategy of combining a reduction in the salt content and a simultaneous modification in the lipid fraction using olive or linseed oils resulted in products with lowest sodium content, highest calcium content and a significant supply of omega-3 fatty acids (García-Iñiguez de Ciriano, Berasategi, Navarro-Blasco, Astiasarán & Ansorena, 2013) and highest MUFA content (Beriaín et al., 2011) producing an improved nutritional sausage profile. Both studies indicated a sensory acceptable characteristic of the new formulations although differences in texture and taste were reported by the trained panel.

The use of starter yeasts can be an alternative to improve the sensory characteristics of the dry fermented sausages. *Debaryomyces hansenii* is the predominant yeast which proliferates in dry fermented sausage environment (Cocolin, Urso, Rantsiou, Cantoni & Comi, 2006). The growth of *D.hansenii* in the sausage surface can control water release in low fat sausages thus improving sausage aroma lost by salt reduction as reported by Campagnol et al. (2011) who tried to improve the sensory characteristic by the addition of a yeast extract (*Saccharomyces cerevisiae*). The mechanisms behind aroma loss due to salt reduction are probably due to the salting out effect that salt produces on volatile compounds (Desmond, 2006) in addition to the effect of salt on the biochemical reactions involved in aroma generation.

Nevertheless, important differences have been observed when different yeasts strains are inoculated in fermented sausages (Olesen, & Stahnke, 2000; Andrade, Córdoba, Sánchez, Casado & Rodríguez, 2009). Recently, Cano-García, Flores &

Belloch (2013) isolated *D. hansenii* strains from traditional fermented sausages and reported their aroma potential using a meat model system (Cano-García, Rivera-Jiménez, Belloch & Flores, 2014). Therefore, the aim of this work was to elucidate the effect of fat and salt reduction on the sensory quality of slow fermented sausages inoculated with *Debaryomyces hansenii*, since this yeast could offset the quality defects produced by fat and/or salt reductions.

2. Materials and methods

2.1. Preparation of yeast inoculum

Debaryomyces hansenii P2 previously isolated from naturally fermented sausages (Cano-García et al., 2013) was used as starter in the production of dry fermented sausages. Yeast was cultivated on GPY medium (2% glucose, 0.5% peptone, 0.5% yeast extract, pH 6.0) and the grown cells washed with sterile saline solution (0.9% NaCl) and centrifuged (7000 rpm for 10 minutes at 4°C) to remove the culture medium. The collected cells were prepared to a concentration of 10^8 c.f.u./ml using dilution plates. The concentrated yeast cells were directly stored at -80°C until their inoculation in dry fermented sausage batches.

2.2. Dry fermented sausages and sampling

Seven batches of dry fermented sausages were manufactured: a control batch (C) was prepared using 70% pork lean meat and 30% pork back fat and 27g/kg NaCl content while six batches were manufactured varying salt and/or pork back fat content with or without yeast inoculation (*Debaryomyces hansenii*). The reformulated batches were: reduced fat (RF); reduced salt (RS); reduced fat and salt (RF+RS); and the same three batches but inoculated with *D. hansenii* yeast (RF+Y, RS+Y, RF+RS+Y). Reduced salt batches were 25 % salt reduced adding 20.25 g/kg NaCl and 6.75 g/Kg KCl. Fat reduced batches were 50 % fat reduced adding 85% lean pork meat and 15% back fat. Appropriate volumes of yeast strain *D. hansenii* P2 suspension were added to the inoculated batches at final concentration of 5×10^6 c.f.u./g of yeast strain. All

fermented sausage batches were produced using the following ingredients: lactose (20 g/kg); dextrin (20g/Kg); sodium caseinate (20 g/kg); glucose (7 g/kg); sodium ascorbate (0.5 g/kg); sodium nitrite (0.15 g/kg); potassium nitrate (0.15 g/kg) and starter culture (0.1 g/kg) SP318 TEXEL SA-301 (Danisco, Cultor, Madrid, Spain) containing *Lactobacillus sakei*, *Pediococcus pentosaceus*, *Staphylococcus carnosus*. The batches were manufactured under the conditions described by Olivares et al. (2010). The meat mixture was kept at 3-5°C for 24h and then was stuffed into collagen casings of 9.5 cm diameter (FIBRAN, S.A., Girona, Spain) being the final weight of each sausage approximately 700 g. The sausages were subjected to drying in a controlled drying chamber at 10-14°C and 70-85% relativity humidity (RH) for 61 days. The weight losses and pH were measured during ripening to control the drying process.

From each batch, 300 g of the meat mixture at 0 days and three sausages at 61 days were randomly chosen for microbial and chemical analysis. From each sample, sausage colour was measured, 20 g were taken for microbial analysis and 150 g were minced and used to measure moisture, water activity and pH. The remaining minced sausages from each batch were vacuum packed and frozen at -20°C till subsequent analysis (fat, protein and ions content). In addition, at 61 days the remaining sausages of each batch were vacuum packaged and stored at 4°C for sensory and texture analysis. All the results were expressed as means of the three replicates per 100 g of dry matter at each processing time and batch.

2.3. Microbial analysis

Sausages samples (20 g) were aseptically homogenized with sterile saline solution (1/10) in a Stomacher (IUL Instruments, Barcelona, Spain) for 1 minute and decimal dilutions were prepared. Lactic acid bacteria population was determined by spread plating on MRS Agar anaerobically (Scharlau Chemie SA, Barcelona, Spain) and staphylococci population by using Mannitol Salt Agar (Scharlau Chemie SA, Barcelona, Spain) both medium were incubated at 37°C for 2 days. Yeast count was

determined in Rose Bengal Agar with chloramphenicol (RBA) (Conda SA, Madrid, Spain) at 28°C for 3 days.

Ten yeast strains isolated from each batch at the initial and final time of the ripening process were subjected to molecular characterization by minisatellite PCR amplification using the M13 primer as described in Cano-García et al (2013). The M13 Minisatellite PCR patterns obtained were compared with the originals previously obtained by Cano-García et al (2013).

2.4. Chemical analysis

The measurement of pH, water activity, colour evaluation (CIELab L*, a*, b*), moisture and fat content was performed as described by Olivares et al. (2010). Nitrogen content was determined by the Kjeldhal method and protein was estimated multiplying the nitrogen content by a factor of 6.25.

Cations (sodium and potassium) and chloride anion were analyzed by ion chromatography as described by Corral et al. (2013). The concentration of each ion was determined by calibration curves using a set of standard solutions of Na⁺, K⁺ and Cl⁻ (Fluka, Switzerland, Sigma, St. Louis, MO). All results were expressed as mg/100g of sample in dry matter.

2.5. Texture profile analysis

Texture profile analysis (TPA) was carried out using TA-XT.plus Texture Analyzer with Texture Exponent software (version 2.0.7.0 Stable Microsystems, Godalming, UK). At the end of the process, three different slices (3.5 cm diameter and 1.5 cm thick) of three sausages from each batch were compressed twice to 50 % of their original height as described by Olivares et al. (2010). The main texture parameters (hardness, springiness and cohesiveness) and the secondary parameter chewiness were obtained from the deformation curves.

2.6. Sensory analysis

Sensory analysis of fermented sausages (61 days) was carried out by 81 untrained assessors. The analysis was made in a sensory laboratory equipped with

individual booths (ISO 8589, 1988). The casing was removed and each sausage was cut into slices of 4mm thickness. One slice of each sausage batch was randomly labelled with three digit codes and presented on a small white plate at room temperature. Water and unsalted bread was provided to clean the palate between samples. A hedonic test was carried out using a 9-hedonic scale labelled on the bottom with “dislike extremely” and on the top “like extremely”. The assessors evaluated their liking of appearance, flavour, taste, hardness, juiciness and overall acceptability. Data acquisition and analysis was performed by Compusense *five* release 5.0 (Compusense Inc., Guelph, ON, Canada).

2.7. Statistical analysis

Analyses of variance (ANOVA) were performed for the chemical and texture parameters to evaluate the differences among samples. A Fisher's test was used to identify significant ($p < 0.5$) differences between types of fermented sausages evaluated. Internal Preference Mapping applied to the mean individual hedonic rates of overall acceptability on all samples was performed. For each product, the coordinates on the preference space determined by the first two components were kept. Then, consumers' hedonic ratings were regressed onto these coordinates, and plotted into the map. Mean values of instrumental parameters were considered as supplementary variables. Clusters analysis was performed to classify consumers according to their preference about dry fermented sausages aroma, taste and overall acceptability. Agglomerative Hierarchical Clustering (AHC) was carried out using Euclidian distance with Ward's method as the aggregation criterion (XLSTAT 2011 Agglomerative hierarchical clustering). A dissimilarity plot was performed to determine how many clusters were suitable for each analysis. A dendrogram was used to define the cluster structure of the data and support the decision that was made using the dissimilarity plot. All statistical analyses were performed using the statistic software XLSTAT 2011 v5.01 (Addinsoft, Barcelona, Spain)

3. Results and discussion

3.1. Chemical analysis

The pH, a_w and weight losses are shown in table 1. The pH showed a reduction in all batches from an initial value of 5.9-6.1 to 4.9-5.3 considered enough to ensure the safety of meat products together with drying and low a_w values (Papadima et al., 1999). In uninoculated batches, RS and RF+RS showed significantly higher pH values than control batch and RF (Table 1) although this has not been previously reported (Liaros et al., 2009; Olivares et al., 2010; Corral et al. 2013). However, the greatest significant differences were found in yeast inoculated batches as all of them presented the highest significant pH probably due to the ability of yeasts to consume organic acids such as lactic acid (Durá, Flores & Toldrá, 2004).

Water activity (a_w) also controlled through the ripening process reached values of 0.90-0.91 thus securing product stability. Few significant differences were observed among batches because the control batch had the lowest a_w (table 1). The effect of salt reduction in a_w values of dry fermented sausages produced different results, no effect was reported in 16 % reduced salt sausages (Corral et al., 2013) while highest a_w values were reported in 50 % reduced salt sausages (Olesen, Meyer & Stahnke, 2004). Moreover fat reduction produced an increase in a_w values (Gómez & Lorenzo, 2013).

Weight losses mainly depend on climatic conditions applied for product ripening (Bloukas et al., 1997). The weight losses in all batches were 39.8-41.6 % at the end the process (table 1). The slow ripening conditions applied during process prevented the effect of fat or/and salt reduction on weight loss (Olivares et al. 2010). However, these processing conditions were not able to avoid the differences ($p<0.05$) found when both reductions were applied together in the inoculated *D. hansenii* batch (RF+RS+Y). The highest weight losses produced by fat reduction have been also reported by other authors (Bloukas et al., 1997; Papadima & Bloukas, 1999; Liaros et al., 2009).

Table 2 shows the chemical composition of dry fermented sausages. A fat reduction of 10-16% was achieved. The moisture content was the highest ($p<0.05$) in

fat reduced batches (Gómez & Lorenzo 2013). However, this effect was not seen ($p>0.05$) in inoculated batches (RF+Y, RS+Y and RF+RS+Y) being in agreement with a_w values obtained. As expected, the highest ($p<0.05$) protein content was found in fat reduced batches due to the highest lean content present as also observed Olivares et al. (2010).

A total sodium reduction of 17-20% was achieved (Table 3). Salt reduced batches both uninoculated and yeast inoculated (RS, RF+RS, RS+Y and RF+RS+Y batches) presented significantly lower Na^+ content and higher K^+ content than no salt reduced batches (C, RF, RF+Y) (Table 3). By contrast, no significant differences were found for Cl^- content among batches since, salt reduced batches were substituted by KCl. Overall, fat or/and salt reduction and *D. hansenii* inoculation did not produced significant differences in colour parameters (L^* , a^* and b^*) (Corral et al., 2013) (data not shown).

3.2. Microbial analysis

LAB and staphylococci are essential for the ripening process and play an important role in the safety and organoleptic characteristics of dry fermented sausages (Ravyts, Vuyst & Leroy, 2012). The levels of LAB, staphylococci and yeast population were analyzed at the beginning and end of the ripening process. At the beginning of the process the mean counts of LAB and staphylococci were 10^6 c.f.u./ g d.m. in all batches while for yeasts 10^7 u.f.c/ g d.m. were found in the inoculated batches. At the end of the process, the levels of LAB and staphylococci were within the range of what could be expected in this product (Table 4) (Durá et al., 2004). The population of LAB experienced a growth of 3-4 logarithmic cycles whilst the population of staphylococci was within the same logarithmic units (Andrade, Córdoba, Casado, Córdoba & Rodríguez, 2010). No significant differences were found among batches for LAB level except the RS+Y batch which presented a significant higher LAB level than inoculated C and RF+RS+Y. However, these results were not correlated to the pH values obtained. Concerning staphylococci growth, all batches with fat and/or salt reduction

had lower staphylococci growth than the control batch. Moreover, *D. hansenii* inoculation did not affect the growth of LAB and neither *Staphylococcus* (table 4). Acidification carried out by LAB causes the inhibition of staphylococci growth (Leroy, Verluyten & De Vuyst, 2006), but in this study the batches which presented the highest pH values were those where the lowest staphylococci counts were found. Therefore, the staphylococci growth could have been affected by fat or/and salt reduction. However, controversial results about their effect on staphylococci have been reported. Several authors reported an absence of effect on staphylococci growth by salt reduction (Corral et al., 2013, Campagnol et al, 2011) while others attributed to KCl the capacity to increase staphylococci growth (Gelabert et al., 2003). With respect to the effect of fat reduction, Del Nobile, Conte, Incoronato, Panza, Sevi & Mariano (2009) reported no effect on staphylococci while Liaros et al. (2009) found a decrease in staphylococci growth in fat reduced sausages attributing it to the lower counts presents in beef and pork meat than in pork back fat. However, Ravyts, Steen, Goemaere, Paelinck, Vuyst & Leroy (2010) reported a limited effect of fat and salt reduction on microbiota growth. In summary, in the present study fat and salt reduction produced a significant decrease on staphylococci growth.

Regarding yeast level at the beginning of the process, all the inoculated batches (RF+Y, RS+Y, RF+RS+Y) showed 10^7 c.f.u./g. yeast while no yeast growth was detected in the uninoculated batches (C, RF, RS, RF+RS). At the end of the process, a low yeast growth was detected in the uninoculated batches (10^4 c.f.u./g) although no differences were detected among batches (Table 4). However, the inoculated batches showed significant higher levels of yeast (10^7 c.f.u./g) than control batches (10^4 c.f.u./g) and this fact was also observed in each respective inoculated versus uninoculated batch.

At the beginning of the process, 100 % of the isolated yeasts from the inoculated batches displayed the original M13 minisatellite pattern of P2 strain indicating the correct inoculation of the batches (Figure 1A Supplementary Material).

Similarly, at the end of the process, all isolated yeasts (100%) showed the same pattern as the strain P2 originally inoculated (Figure 1B, Supplementary Material). These results demonstrate that *D. hansenii* P2 was able to survive and replicate in the sausage environment even with fat and/or salt reduction, confirming the dominance of the inoculated P2 *D. hansenii* along the ripening process. In the case of the control batch, solely 20 % of the M13 patterns corresponded to P2 (Figure 1C, Panel C, Supplementary Material). However, the percentage increased between 60% -70% in the uninoculated batches with salt and fat reduction (Figure 1C, Panels RS and RF, Supplementary Material) and reached 90 % in the uninoculated batch with both salt and fat reduction (Figure 1C, Panel RS+RF, Supplementary Material). The presence of *D. hansenii* strains in the uninoculated sausage batches was probably due to the dispersion of the inoculated yeasts along the 61 days of ripening favoured by air circulation in the drying chamber. Nevertheless, the non-inoculated batches presented lower *D. hansenii* P2 counts than the inoculated ones.

3.3. Texture profile analysis

TPA parameters were analyzed in the final product (Table 5). Hardness and consequently chewiness were affected by the different formulations whilst no effect was observed on springiness and cohesiveness. In uninoculated batches, RF, RS and RF+RS showed significant higher hardness and chewiness than control batch. Moreover, RS batch showed the highest hardness and chewiness. On the contrary, in inoculated batches, only RF+Y and RF+RS+Y batches showed significant higher hardness than the control batch. The effect of the inoculated *D. hansenii* yeast was significant in salt reduced batches (RS and RS+Y) as it produced a decrease in the hardness and chewiness. However, springiness and cohesiveness were not affected by neither formulation nor *D. hansenii* inoculation. This increase in hardness and chewiness has been already reported in dry fermented sausage when fat was reduced (Olivares et al., 2010). However, salt effect on fermented sausage texture is

contradictory as generally low changes in texture have been reported when KCl was used as unique salt substitute (Gou, Guerrero, Gelabert & Arnau, 1996) or a decrease in sausage hardness (Gimeno et al. 1999); although any reference has indicated an increase in hardness as observed in our uninoculated sausages.

3.4. Sensory analysis

An Internal Preference Mapping was done with mean scores of overall acceptability and the following supplementary parameters which showed significant differences between batches: pH, a_w , weight losses, ions content (Na^+ , K^+ , Cl^-), fat, protein and moisture content, microbiological analysis (LAB, Staphylococcus and yeast counts), texture analysis (hardness, chewiness) and consumer liking (appearance, aroma and taste) (Figure 1). Two principal components were able to explain the 42.8% of the total variance. PC1 accounted for 22.9% of the variance and distinguished samples according to consumer preferences placing C, RF+RS and inoculated samples on the right quadrants and salt (RS) or fat (RF) reduced samples on the left quadrants. However, PC2 accounted for 19.9% of the variance and distinguished samples by the presence of yeast (in the positive part of the axe are placed samples without yeast inoculation and in the negative part of the axe inoculated samples). Taking into account supplementary parameters plotted, C, RF+RS and RF+Y samples were related with moisture, fat and Na^+ and Cl^- content, staphylococci counts and taste liking; RS and RF samples were related with texture parameters and RS+Y, RF+RS+Y samples were related with pH, protein and potassium content and yeast.

For a better understanding of consumer responses, the preferences for attributes that showed significant differences (taste and overall acceptability) plus the attribute aroma were also analyzed by cluster analysis using Euclidean distances (Figure 2). The attribute aroma was analyzed due to the effect of yeast on sausage aroma (Cano-García et al., 2013). The number of consumers in each cluster was different and depended on the analyzed attribute, thirty-nine and forty-two consumers for aroma, thirty-four and twenty-seven for taste and fifty and thirty-one for overall

acceptability, in cluster 1 and 2 respectively. The sausage preference of each cluster was elucidated by one-way ANOVA. Based on aroma, taste and overall acceptability, cluster 1 preferred fat reduced sausages without yeast inoculation while cluster 2 preferred inoculated and fat reduced sausages (Figure 2).

Salt reduced sausage (RS) was perceived less tasty and the cluster 2 also perceived it less aromatic. Although RS was overall accepted by cluster 2, cluster 1 did not sensory accepted it. Moreover, the yeast inoculation on salt reduced sausages (RS+Y) was accepted better than uninoculated salt reduced sausages by both clusters, even though the sausage aroma and taste did not significantly improve by yeast inoculation. This fact agree with those reported by Corral et al. (2013) and Aaslyng et al., (2014) who pointed out that consumers consider reduced sausages an acceptable product although the sensory characteristic are affected. Also, other studies have reported an improvement of sausage aroma and taste using yeast extracts (Campagnol et al., 2011).

Fat reduced sausage was sensory accepted by the two consumer clusters; although, cluster 2 perceived it less tasty and aromatic. However, the yeast inoculation on fat reduction sausage was perceived with more sausage taste and aroma by cluster 2. This result agrees with Olivares et al. (2011) who reported a lower aroma in low fat sausages although Liaros et al. (2009) and Papadima et al. (1999) reported no effect of fat reduction on sausage odour or taste.

When the salt and fat reduction was carried out together, the two clusters perceived this sausage the less tasty than the other formulations, and cluster 1 even perceived it less aromatic. In this case, the yeast inoculation did not improve the sausage taste or aroma.

Nevertheless, different preferences patterns of consumers were found; the yeast inoculation improved the aroma and taste quality when the fat or salt reductions were carried out in dry fermented sausages. Several authors have studied the effect of *D. hansenii* on sausage aroma reporting an increase in some volatile compounds

(Andrade et al., 2010) while Olesen & Stahnke (2000) reported few differences between control and yeast inoculated sausages. However, there are not reports about the effect *D. hansenii* inoculation on consumer acceptability and when fat or salt is reduced. In this sense, the addition of yeast extracts produced an increase in the aroma acceptability of sausages (Bolumar, Sanz, Flores, Aristoy, Toldrá & Flores 2006, and Campagnol et al., 2011) while only Flores, Durá, Marco & Toldrá (2004) confirmed the beneficial effect of the inoculation of *D. hansenii* in fermented sausages on consumer aroma acceptability. However, they did not confirm the implantation of the inoculated yeast strain. In addition, further studies are necessary to elucidate the biochemical process involved in the improvement of quality and aroma in low salt and fat dry fermented sausages by the inoculation of *D. hansenii* yeast.

4. Conclusion

The inoculation of *D. hansenii* yeast on salt and fat reduced sausages was able to compensate the changes in a_w and texture although it was not able to modify neither the hardness of reduced fat batches nor the decrease in staphylococci growth. In terms of sensory analysis, yeast inoculation improved the aroma and taste quality when fat or salt reductions were done. However, when salt and fat reduction was carried out together, yeast inoculation did not improve sausage taste or aroma. Further studies are necessary to elucidate the biochemical process involved in aroma generation and the interactions with salt and fat reductions.

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References

- Aaslyng, M. D., Vestergaard, C., & Koch, A. G. (2014). The effect of salt reduction on sensory quality and microbial growth in hotdog sausages, bacon, ham and salami. *Meat Science*, 96, 47-55.
- Andrade, M. J., Córdoba, J. J., Casado, E. M., Córdoba, M. G., & Rodríguez, M. (2010). Effect of selected strains of *Debaryomyces hansenii* on the volatile compound production of dry fermented sausage "salchichón". *Meat Science*, 85, 256-264.
- Andrade, M.J., Córdoba, J.J., Sánchez, B., Casado, E.M., & Rodríguez, M. (2009). Evaluation and selection of yeasts isolated from dry-cured Iberian ham by their volatile compound production. *Food Chemistry*, 113, 457-463.
- Beriain, M. J., Gómez, I., Petri, E., Insausti, K., & Sarriés, M. V. (2011). The effects of olive oil emulsified alginate on the physico-chemical, sensory, microbial, and fatty acid profiles of low-salt, inulin-enriched sausages. *Meat Science*, 88, 189-197.
- Bloukas, J. G., Paneras, E. D., & Fournitzis, G. C. (1997). Effect of replacing pork backfat with olive oil on processing and quality characteristics of fermented sausages. *Meat Science*, 45, 133-144.
- Bolumar, T., Sanz, Y., Flores, M., Aristoy, M. C., Toldrá, F., & Flores, J. (2006). Sensory improvement of dry-fermented sausages by the addition of cell-free extracts from *Debaryomyces hansenii* and *Lactobacillus sakei*. *Meat Science*, 72, 457-466.

417 Cano-García, L., Flores, M., & Belloch, C. (2013). Molecular characterization and
 418 aromatic potential of *Debaryomyces hansenii* strains isolated from naturally
 419 fermented sausages. *Food Research International*, 52, 42-49.

420 Cano-García L, Rivera-Jiménez S, Belloch C and Flores M. Generation of aroma
 421 compounds in a fermented sausage meat model system by *D. hansenii* strains.
 422 (2014) *Food Chemistry*, 151, 364-373.

423 Campagnol, P. C. B., dos Santos, B. A., Wagner, R., Terra, N. N., & Pollonio, M. A. R.
 424 (2011). The effect of yeast extract addition on quality of fermented sausages at
 425 low NaCl content. *Meat Science*, 87, 290-298.

426 Corral, S., Salvador, A., & Flores, M. (2013). Salt reduction in slow fermented
 427 sausages affects the generation of aroma active compounds. *Meat Science*, 93,
 428 776-785.

429 Cocolin, L., Urso, R., Rantsiou, K., Cantoni, C. & Comi, G. (2006). Dynamics and
 430 characterization of yeasts during natural fermentation of Italian sausages.
 431 *FEMS Yeast Research*, 6, 692–701.

432 Del Nobile, M.A., Conte, A., Incoronato, A.L., Panza, O, Sevi, A.,& Mariano, R. (2009).
 433 New strategies for reducing the pork back-fat in typical Italian salami. *Meat*
 434 *Science*, 81, 263-269.

435 Desmond, E. (2006). Reducing salt: A challenge for the meat industry. *Meat Science*,
 436 74, 188-196.

437 Durá, M. A., Flores, M., & Toldrá, F. (2004). Effect of *Debaryomyces* spp. on the
 438 proteolysis of dry-fermented sausages. *Meat Science*, 68(2), 319-328.

439 Flores, M., Durá, M. A., Marco, A., & Toldrá, F. (2004). Effect of *Debaryomyces* spp. on
 440 aroma formation and sensory quality of dry-fermented sausages. *Meat Science*,
 441 68, 439-446.

442 García-Íñiguez de Ciriano, M., Berasategi, I., Navarro-Blasco, I., Astiasarán, I., &
 443 Ansorena, D. (2013). Reduction of sodium and increment of calcium and ω -3
 444 polyunsaturated fatty acids in dry fermented sausages: Effects on the mineral

445 content, lipid profile and sensory quality. *Journal of the Science of Food and*
 446 *Agriculture*, 93, 876-881.

447 Gelabert, J., Gou, P., Guerrero, L., & Arnau, J. (2003). Effect of sodium chloride
 448 replacement on some characteristics of fermented sausages. *Meat Science*, 65,
 449 833-839.

450 Gimeno, O., Astiasarán, I., & Bello, J. (1999). Influence of partial replacement of NaCl
 451 with KCl and CaCl₂ on texture and color of dry fermented sausages. *Journal of*
 452 *Agricultural and Food Chemistry*, 47, 873-877.

453 Gómez, M., & Lorenzo, J. M. (2013). Effect of fat level on physicochemical, volatile
 454 compounds and sensory characteristics of dry-ripened “chorizo” from Celta pig
 455 breed. *Meat Science*, 95, 658-666.

456 Gou, P., Guerrero, L., Gelabert, J., & Arnau, J. (1996). Potassium chloride, potassium
 457 lactate and glycine as sodium chloride substitutes in fermented sausages and in
 458 dry-cured pork loin. *Meat Science*, 42, 37-48.

459 ISO 8589 (1988). Sensory analysis. General guidance for design of test rooms.
 460 Standard no.8589 (Geneva, Switzerland).

461 Leroy, F., Verluyten, J., & De Vuyst, L. (2006). Functional meat starter cultures for
 462 improved sausage fermentation. *International Journal of Food Microbiology*,
 463 106, 270-285.

464 Liaros, N. G., Katsanidis, E., & Bloukas, J. G. (2009). Effect of the ripening time under
 465 vacuum and packaging film permeability on processing and quality
 466 characteristics of low-fat fermented sausages. *Meat Science*, 83, 589-598.

467 Mendoza, E., García, M. L., Casas, C., & Selgas, M. D. (2001). Inulin as fat substitute
 468 in low fat dry fermented sausages. *Meat Science*, 57, 387-393.

469 Muguerza, E., Ansorena, D., & Astiasarán, I. (2003). Improvement of nutritional
 470 properties of Chorizo de Pamplona by replacement of pork backfat with soy oil.
 471 *Meat Science*, 65, 1361-1367.

472 Olesen, P. T., Meyer, A. S., & Stahnke, L. H. (2004). Generation of flavour compounds
 473 in fermented sausages - The influence of curing ingredients, *Staphylococcus*
 474 starter culture and ripening time. *Meat Science*, 66, 675-687.

475 Olesen, P. T., & Stahnke, L. H. (2000). The influence of *Debaryomyces hansenii* and
 476 *Candida utilis* on the aroma formation in garlic spiced fermented sausages and
 477 model minces. *Meat Science*, 56, 357-368.

478 Olivares, A., Navarro, J. L., & Flores, M. (2011). Effect of fat content on aroma
 479 generation during processing of dry fermented sausages. *Meat Science*, 87,
 480 264-273.

481 Olivares, A., Navarro, J. L., Salvador, A., & Flores, M. (2010). Sensory acceptability of
 482 slow fermented sausages based on fat content and ripening time. *Meat*
 483 *Science*, 86, 251-257.

484 Papadima, S. N., & Bloukas, J. G. (1999). Effect of fat level and storage conditions on
 485 quality characteristics of traditional Greek sausages. *Meat Science*, 51, 103-
 486 113.

487 Ravyts, F., Vuyst, L. D., & Leroy, F. (2012). Bacterial diversity and functionalities in
 488 food fermentations. *Engineering in Life Sciences*, 12, 356-367.

489 Ravyts, F., Steen, L., Goemaere, O., Paelinck, H., De Vuyst, L., & Leroy, F. (2010).
 490 The application of staphylococci with flavour-generating potential is affected by
 491 acidification in fermented dry sausages. *Food Microbiology*, 27, 945-954.

492 Ruiz-Capillas, C., Triki, M., Herrero, A. M., Rodriguez-Salas, L., & Jiménez-Colmenero,
 493 F. (2012). Konjac gel as pork backfat replacer in dry fermented sausages:
 494 Processing and quality characteristics. *Meat Science*, 92, 144-150.

495 Ruusunen, M. & Puolanne, E. (2005). Reducing sodium intake from meat products.
 496 *Meat Science*, 70, 531-541.

497 Salazar, P., García, M. L., & Selgas, M. D. (2009). Short-chain fructooligosaccharides
 498 as potential functional ingredient in dry fermented sausages with different fat
 499 levels. *International Journal of Food Science and Technology*, 44, 1100-1107.

500 WHO/FAO Expert Consultation (2003). *Diet, nutrition and the prevention of chronic*
501 *diseases*. WHO technical report series 916, Geneva.
502 Wirth, F. (1988). Technologies for making fat-reduced meat products. *Fleischeirtsch*,
503 *68*, 1153-1156.
504

FIGURA LEGENDS

Figure 1. Loadings of the first two principal components (PC1-PC2) of consumer acceptability (black square), samples (black circles) and instrumental parameters (grey rhombus).

Figure 2. Mean values of the A) aroma; B) taste; C) overall acceptability by consumer cluster. Different letters in each cluster indicate significant differences at $p < 0.05$.

Figure 1 Supplementary data. Electrophoretic patterns of minisatellite M13 PCR amplification of strains isolated at 0 d (A) and 61 days (B, C). In each photograph: first lane represents “M” 100 pb ladder (Invitrogen, Carlsbad, CA, USA) followed by the original inoculated P2 yeast (P2) and the 10 isolated strains in the sausage batches. Sausage batches; Control (C), reduced fat (RF), reduced salt (RS), reduced salt and fat (RS+RF) and inoculated reduced fat (RF+Y), inoculated reduced salt (RS+Y) and inoculated reduced salt and fat (RS+RF+Y).

Table 1. Effect of salt and fat reduction on pH, aw and weight losses of dry fermented sausages inoculated with *D. hansenii* yeast.

Table 2. Effect of salt and fat reduction on chemical composition of dry fermented sausages inoculated with *D. hansenii* yeast.

Table 3. Effect of salt and fat reduction on ions content in dry fermented sausages inoculated with *D. hansenii* yeast.

Table 4. Effect of salt and fat reduction on Lactic acid bacteria, Staphylococci and yeast counts in dry fermented sausages inoculated with *D. hansenii* yeast.

Table 5. Effect of salt and fat reduction on texture parameters in dry fermented sausages inoculated with *D. hansenii* yeast.

Table 1. Effect of salt and fat reduction on pH, aw and weight losses of dry fermented sausages inoculated with *D. hansenii* yeast.

	pH		aw		Weight losses (%)	
	Uninoculated	Inoculated	Uninoculated	Inoculated	Uninoculated	Inoculated
C	5.0 Ab	5.0 Ab	0.906 Ab	0.906 Aa	39.8 Aa	39.8 Ab
RF	4.9 Bb	5.2 Aa	0.914 Ba	0.916 Aa	40.6 Aa	39.8 Ab
RS	5.1 Ba	5.2 Aa	0.912 Aa	0.912 Aa	41.2 Aa	40.6 Ab
RF+RS	5.1 Ba	5.3 Aa	0.916 Aa	0.913 Aa	40.0 Ba	41.6 Aa

Different small letters in the same column indicate significant differences at $P < 0.05$.

Different capital letters in each row for each parameter indicate significant differences at $P < 0.05$.

Table 2. Effect of salt and fat reduction on chemical composition of dry fermented sausages inoculated with *D. hansenii* yeast.

	Moisture (%)		Fat (%)		Protein (%)	
	Uninoculated	Inoculated	Uninoculated	Inoculated	Uninoculated	Inoculated
C	46.0 Ac	46.0 Aa	17.5 Aa	17.5 Aa	33.3 Ac	33.3 Ab
RF	46.9 Ab	47.3 Aa	15.3 Abc	15.7 Aab	34.5 Ab	35.4 Aa
RS	46.2 Ac	46.3 Aa	16.7 Aab	16.6 Aa	34.7 Ab	34.9 Aab
RF+RS	47.6 Aa	46.4 Aa	15.0 Ac	14.7 Ab	35.2 Aa	35.9 Aa

Different small letters in the same column indicate significant differences at $P < 0.05$.

Different capital letters in each row for each parameter indicate significant differences at $P < 0.05$.

Table 3. Effect of salt and fat reduction on ions content in dry fermented sausages inoculated with *D. hansenii* yeast.

	Na ⁺ (mg/100g d.m.)		K ⁺ (mg/100g d.m.)		Cl ⁻ (mg/100g d.m.)	
	Uninoculated	Inoculated	Uninoculated	Inoculated	Uninoculated	Inoculated
C	2925.6 Ab	2925.6 Aa	968.2 Ab	968.2 Ac	4261.8 Aa	4261.8 Aa
RF	3078.1 Aa	3032.7 Aa	1024.8 Ab	1064.7 Ab	4127.2 Aa	4207.4 Aa
RS	2527.5 Ac	2473.6 Ab	1937.6 Aa	1910.7 Aa	4176.7 Aa	4054.9 Aa
RF+RS	2553.9 Ac	2495.0 Ab	1980.9 Aa	1969.0 Aa	4176.8 Aa	4181.3 Aa

Different small letters in the same column indicate significant differences at $P < 0.05$.

Different capital letters in each row for each parameter indicate significant differences at $P < 0.05$.

Table 4. Effect of salt and fat reduction on Lactic acid bacteria, Staphylococci and yeast counts in dry fermented sausages inoculated with *D. hansenii* yeast.

	LAB (cfu/g dm)				Staphylococci (cfu/g dm)				Yeast (cfu/g dm)			
	Uninoculated		Inoculated		Uninoculated		Inoculated		Uninoculated		Inoculated	
C	2.7E+09	Aa	2.7E+09	Ab	1.7E+07	Aa	1.7E+07	Aa	3.2E+04	Aa	3.2E+04	Ab
RF	2.6E+09	Aa	7.4E+09	Aab	2.3E+06	Ab	1.7E+06	Ab	1.8E+04	Ba	5.5E+07	Aa
RS	3.7E+09	Aa	12.1E+09	Aa	3.1E+06	Ab	1.6E+06	Ab	1.3E+04	Ba	2.7E+07	Aa
RF+RS	2.8E+09	Aa	4.3E+09	Ab	2.0E+06	Ab	1.9E+06	Ab	1.6E+04	Ba	4.1E+07	Aa

Different small letters in the same column indicate significant differences at $P < 0.05$.

Different capital letters in each row for each parameter indicate significant differences at $P < 0.05$.

Table 5. Effect of salt and fat reduction on texture parameters in dry fermented sausages inoculated with *D. hansenii* yeast.

	Hardness		Springiness		Cohesiveness		Chewiness	
	Uninoculated	Inoculated	Uninoculated	Inoculated	Uninoculated	Inoculated	Uninoculated	Inoculated
C	172.7 Ac	172.7 Ac	0.69 Aa	0.69 Aa	0.66 Aa	0.66 Aa	77.4 Ac	77.4 Aa
RF	203.9 Aab	200.7 Aa	0.66 Aa	0.67 Aa	0.65 Aa	0.65 Aa	88.5 Aab	87.3 Aa
RS	217.2 Aa	178.4 Bbc	0.67 Aa	0.71 Aa	0.65 Aa	0.64 Aa	95.4 Aa	80.9 Ba
RF+RS	201.8 Ab	192.7 Aab	0.66 Aa	0.68 Aa	0.65 Aa	0.65 Aa	86.6 Ab	85.2 Aa

Different small letters in the same column indicate significant differences at $p < 0.05$. Different capital letters in each row for each parameter indicate significant differences at $p < 0.05$.

Figure 1
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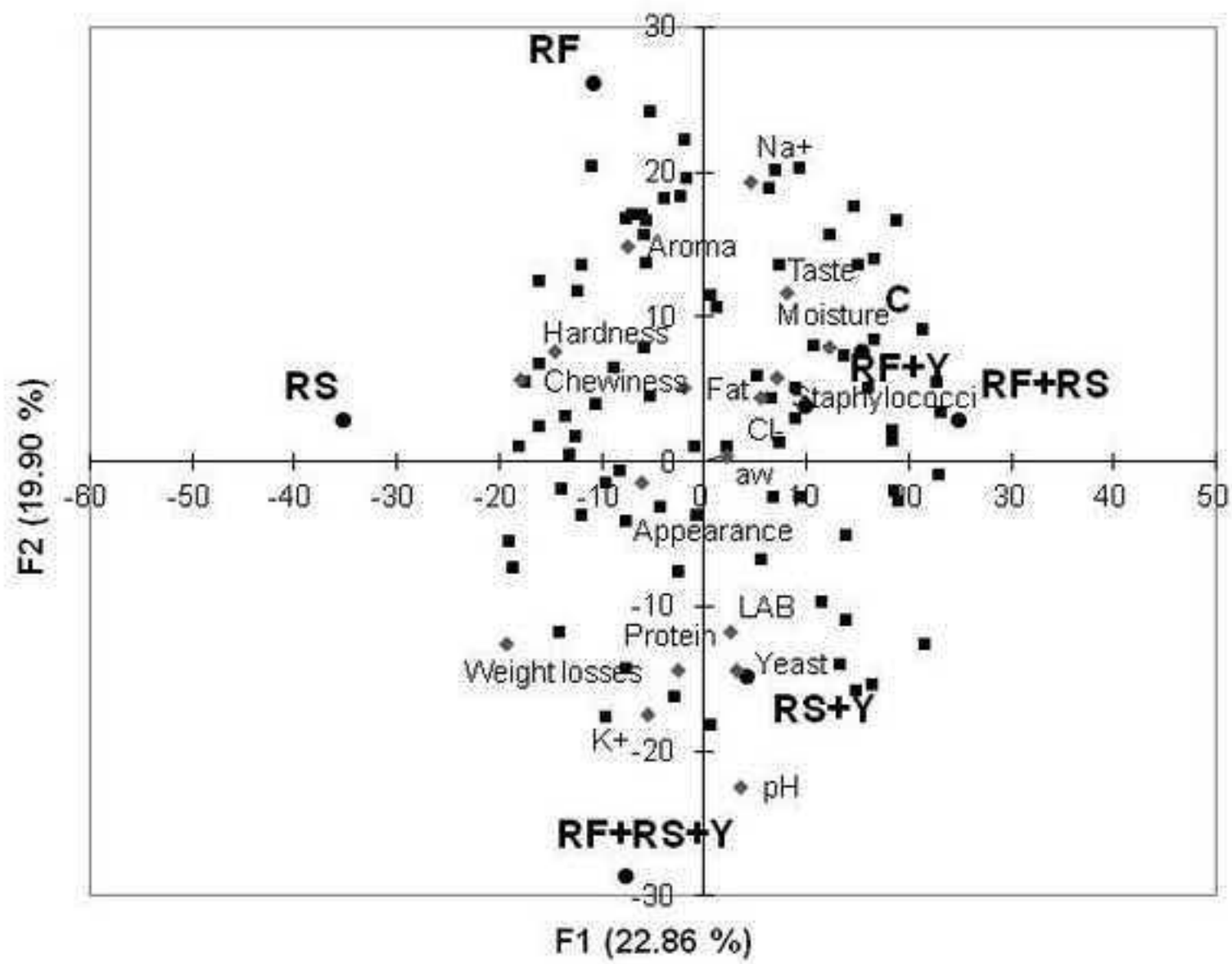


Figure 2
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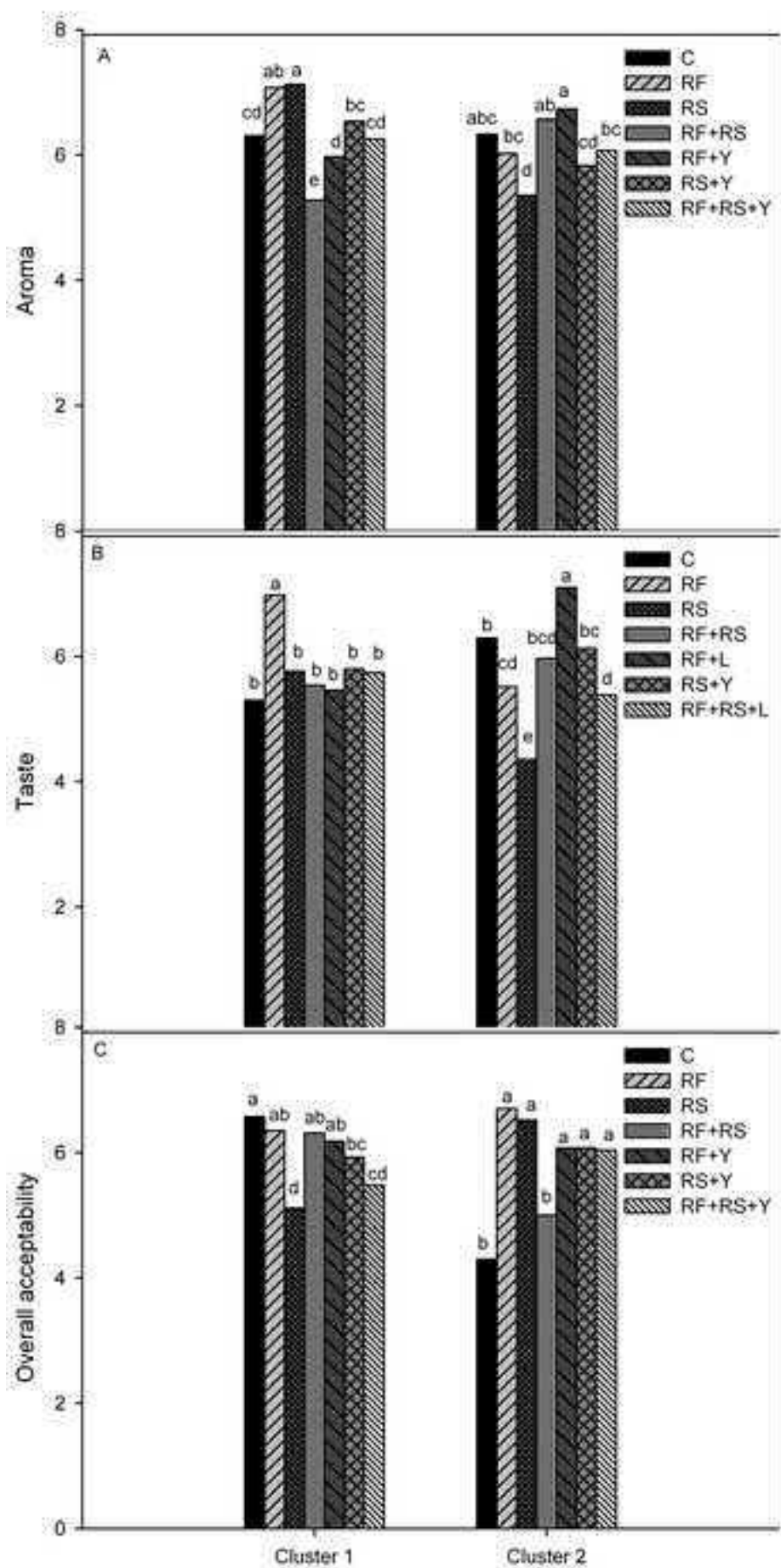
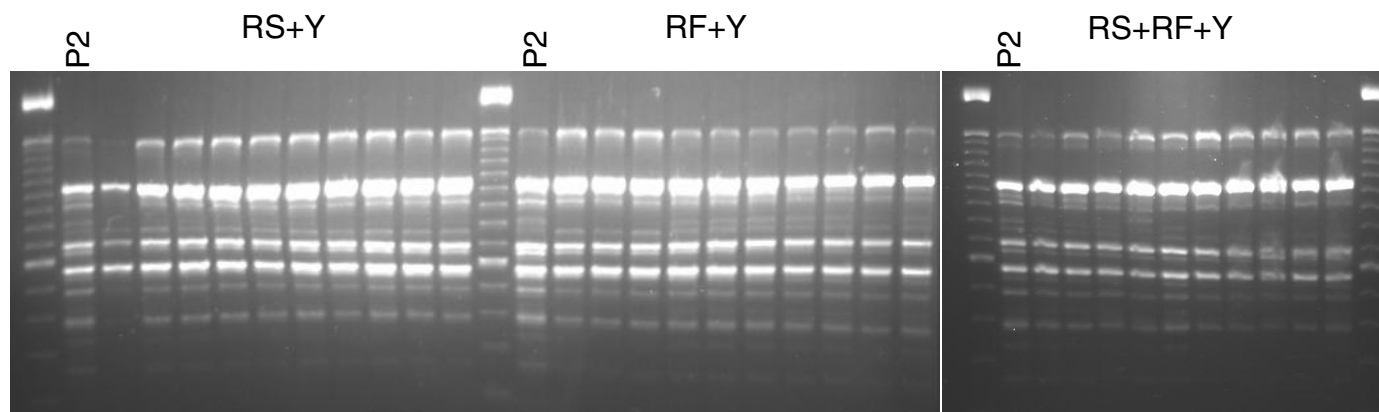
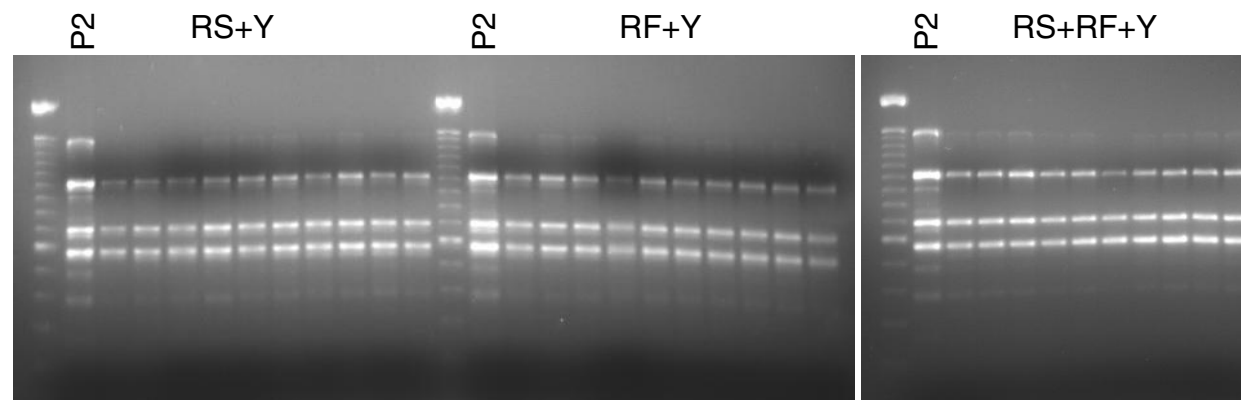


Figure supplementary

A) Time 0 days



B) Time 61 days



C) Time 61 days

